Metabolic Activation of PCBs to Quinones: Reactivity toward N and S Nucleophiles

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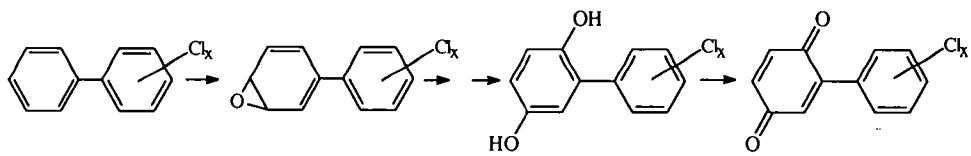
1. Introduction

Metabolism of xenobiotics often involves an hydroxylation reaction (the introduction of an OH functionality into the molecule) followed by conjugation with an endogenous moiety, such as glutathione, an amino acid or a sugar. The net result of this process is generally a detoxified xenobiotic that is both water-soluble and easily excretable. Occasionally, the metabolism of foreign compounds produces electrophilic intermediates and metabolites, which are capable of binding to critical cellular targets, such as proteins and DNA.

Aromatic hydrocarbons, including halogenated benzenes and halogenated biphenyls, frequently undergo cytochrome P-450 catalyzed hydroxylation reactions, which may lead to reactive intermediates, such as an arene oxide, depicted as **A** in the scheme below. When two introduced hydroxyl groups are ortho or para to each other, oxidation to a quinone may be catalyzed by one of several peroxidases present within the cell. Quinones are themselves reactive electrophiles, an example is designated as **B** below:



A B

The goal of the present study was to investigate the occurrence of dihydroxy metabolites of polychlorinated biphenyls (PCBs), their oxidation to quinones and the reactivity of these PCB metabolites toward N and S nucleophiles.

2. Materials and Methods

(Dihydroxybiphenyl and Quinone Synthesis) We have reported the synthesis, isolation, and

characterization of the chlorinated dihydroxybiphenyls[1]. The chlorophenyl-substituted-1,4-benzoquinones were synthesized using the Meerwein arylation as described[2].

(Metabolism) The final incubation medium consisted of 25 mM Tris-HCl buffer (pH 8.0), 2 mM $MgCl_2$, 2 mg microsomal protein/mL and a regenerating system consisting of 5 mM glucose-6-phosphate, 0.5 mM $NADP^+$ and 0.75 units/mL glucose-6-phosphate dehydrogenase. After a 5 min preincubation, 4-chlorobiphenyl, dissolved in DMSO, was added to a final concentration of 1 mM (0.5% DMSO). Incubations (total volume 2 mL) were carried out for 20 min at 37 °C in a shaking water bath and were stopped by adding HCl to 0.36 M. NaCl was added and the metabolites were extracted with diethyl ether. The combined extracts were dried and the resulting residue stored in the dark until GC and GC/MS analysis.

(Oxidation of 2',5'-dihydroxy-4-chlorobiphenyl) 2',5'-Dihydroxy-4-chlorobiphenyl, dissolved in DMSO, was pipetted into cuvettes containing 50 mM Tris-HCl buffer (pH 8.0), and horseradish peroxidase (type 6). This enzyme was employed as a model for the several mammalian peroxidases which catalyze this oxidation. After recording a baseline curve, incremental additions of 1 mM H_2O_2 were carried out and the absorption spectrum recorded.

(Reactivity with Nucleophiles) A. Kinetic analysis of amino acid addition to 2-(4'-chlorophenyl)-1,4-benzoquinone. The rates of various amino acids were determined following the decrease in absorbance of the 2-(4'-chlorophenyl)-1,4-benzoquinone chromophore at 380 nm (ε = 1430 M^{-1} cm^{-1}). Reactions, run under pseudo first- order conditions, were initiated by adding 5 μL of a 25 mM solution of the quinone in DMSO to 995 μL of a buffer solution (500 μL of phosphate buffer, 495 μL of amino acid, pH 7.4) in a quartz cuvette. The absorbance of each reaction was monitored on a Shimadzu MPS-2000 UV-vis spectrophotometer at 25 °C for 15 minutes. All plots of ln A_t versus time were linear, indicating that each reaction followed pseudo first-order kinetics. Pseudo first-order rate constants were estimated for each reaction from the slope of the regression lines fit to each plot. Rate constants were normalized to second-order rate constants with units of min^{-1} M^{-1} by dividing by the amino acid concentration (12 mM).

B. Analysis of nucleophilic addition of thiols to 2-(4'-chlorophenyl)-1,4-benzoquinone and subsequent reoxidation of the thiol-hydroquinone adducts. Reactions were run under second-order conditions with the addition of 10 μL of 25 mM glutathione or N-acetyl-L-cysteine to 10 μL of 25 mM 2-(4'-chlorophenyl)-1,4-benzoquinone in 970 μL of 50 mM phosphate buffer solution. The reaction was monitored over the range of 650 nm to 350 nm and was recorded using the Shimadzu MPS-2000 UV-vis spectrophotometer. Horseradish peroxidase (5 μL of 0.25 units/μL) and 0.1 M H_2O_2 (5 μL) were added to the cuvette and the reaction was monitored over the same wavelength range and recorded.

3. Results and Discussion

(Metabolism)

When 4-chlorobiphenyl was incubated with liver microsomes from rats treated with phenobarbital, 3-methylcholanthrene or a combination of the two, three diol metabolites were formed. These represent the three possible one ring dihydroxy metabolites (catechols and hydroquinones) which can be oxidized to quinones. The major metabolite in each microsomal incubation was the -3',4'-diol, followed by the -2',3'-diol, and the -2',5',-diol (Table 1).

Table 1. Formation of diol metabolites from 4-chlorobiphenyl during a 20 minute incubation with microsomes from rats treated with either phenobarbital (PB), 3-methylcholanthrene (3-MC) or the combination (PB + 3-MC).

	Amount of Diol Metabolite Formed[a]		
Inducer	2',3'-Diol	2',5'-Diol	3',4'-Diol
Phenobarbital	nd[b]	6.51 ± 1.0	13.9 ± 1.0
3-Methylcholanthrene	19.1 ± 0.6	10.6 ± 0.2	22.9 ± 2.3
PB + 3-MC	18.9 ± 1.6	10.6 ± 1.3	34.5 ± 5.1

[a]nmol metabolite; means ± standard deviation; n=4
[b]nd = none detected

(Oxidation of Metabolites)

Extracted metabolites of 4-chlorobiphenyl (and other lower halogenated biphenyls) may be oxidized using horseradish peroxidase and H_2O_2 to products which have characteristic visible absorption maxima. The formation of these compounds may be followed spectrometrically. In Figure 1 the stepwise conversion of 2',5'-dihydroxy-4-chlorobiphenyl to the corresponding quinone is seen, as limiting increments of H_2O_2 are sequentially added. The primary hydroquinone absorbance at 300 nm diminishes in a stepwise fashion as the quinone absorbance at 380 nm increases. After all of the hydroquinone is oxidized, an additional peak at approximately 415 nm appears which corresponds to Compound I of horseradish peroxidase. The oxidation of the hydroquinone is therefore dependent on both the enzyme and the oxidant.

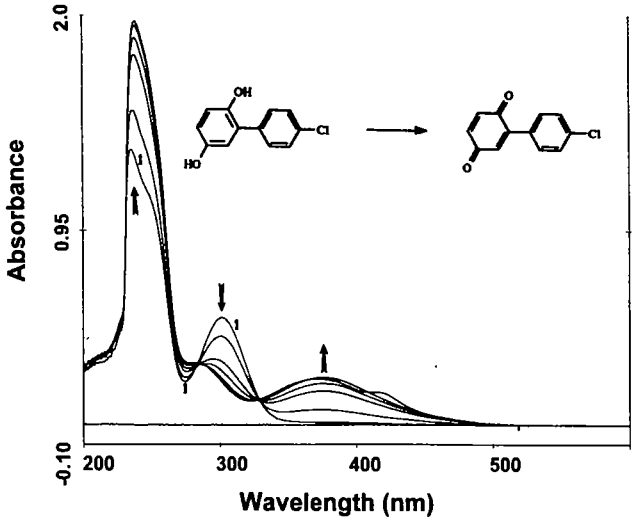
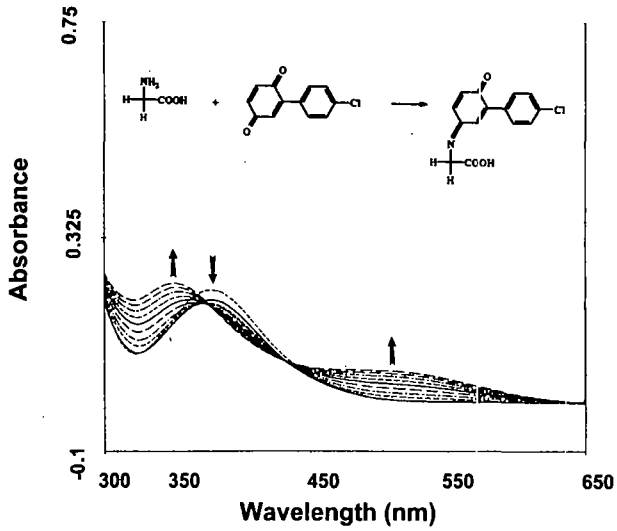


FIGURE 1

(Reactivity with Nucleophiles)

The nitrogen nucleophiles included L-arginine, glycine, L-histidine and L-lysine. The rate of reactivity of each nucleophile with 2-(4'- chlorophenyl) -1,4- benzoquinone was determined by following the disappearance of absorbance at 380 nm. All reactions were conducted in the presence of a 99-fold excess of each amino acid, and all reactions followed pseudo first-order kinetics. The rate constants for each reaction ranged between 0.45 - 0.75 min^{-1} M^{-1}. These data suggest that although the rate of each reaction is rather slow at pH 7.4 the PCB quinones will still react with nitrogen nucleophiles. The UV-vis spectrum of the reaction of 2-(4'-chlorophenyl)-1,4-benzoquinone and glycine is shown in Figure 2. The reaction was monitored every five minutes for 45 minutes. The pH of the reaction was 9.0, a pH at which the absorbance changes were more pronounced. The isosbestic point near 430 nm indicates that only 2 principal species are involved. The original quinone absorbance (380 nm) slowly decreases over time while there is a gradual hypsochromic shift from 380 nm to 340 nm. A new absorbance also appears at about 500 nm which is attributed to the formation of a new product, a PCB-glycine conjugate. We have assigned an imine structure to the product because similar reactions with methyl substituted 1,4-benzoquinones and aromatic amines, such as benzidine, have also produced imine products[3,4]. In the following paper we show that nucleotide bases such as adenine and guanine react with PCB-quinone metabolites.

FIGURE 2



The reaction of glutathione (GSH) and N-acetyl-L-cysteine (NAC) with the quinones was also studied. A rate could not be calculated under either pseudo first-order or second-order conditions because the loss of quinone absorbance was instantaneous upon the addition of GSH or NAC. However, the UV-vis spectra in Figure 3 provide some insight into the reactivity of thiols with PCB quinone-derived metabolites. The addition of glutathione to the quinone is

represented in Spectrum B. The quinoid absorbance at 380 nm (Figure 3, Spectrum A) completely disappears and a new absorbance appears at 310 nm; this absorbance is most likely due to hydroquinone-glutathione adducts. Hydroquinone-glutathione adducts reported in the literature also show absorbances in the same region of the UV-vis spectrum[5-8]. When the products from Spectrum B were oxidized with horseradish peroxidase and H_2O_2, there was a complete loss of absorbance at 310 nm, while a new absorbance appeared at 365 nm (Figure 3, Spectrum C). The absorbance at 365 nm in Spectrum C represents the formation of quinone-glutathione adducts via the horseradish peroxidase and H_2O_2-catalyzed re-oxidation of the hydroquinone. This is a significant finding because it suggests that the hydroquinone-glutathione adducts formed after 1,4-Michael addition are themselves susceptible to oxidation. This further oxidation of the hydroquinone-glutathione adducts could result in a depletion of glutathione levels or, alternatively, the PCB-glutathione conjugates may participate in redox cycling. A recent review on the toxicology of quinone-thioethers has shown that many of these compounds are nephrotoxic[9]. It remains to be determined if (PCB-Quinone)-glutathione adducts will demonstrate this characteristic.

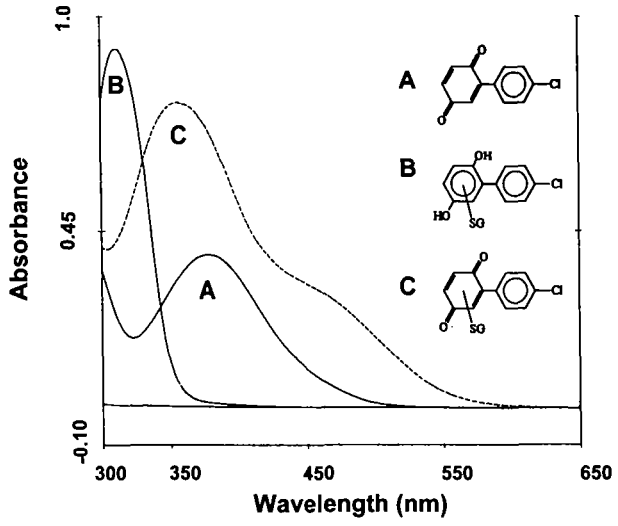


FIGURE 3

In conclusion, our studies clearly show that: A. PCBs are subject to attack by microsomal enzymes giving rise to metabolites which may be activated to electrophiles, B. Specifically, metabolites possessing OH functionalities ortho and para to each other (catechols and hydroquinones) may be oxidized to quinones, and C. PCB-derived quinones may react with nitrogen and sulfur nucleophiles at physiological pH. This knowledge of the characteristics of PCB metabolites will aid in our understanding of the mechanisms by which PCBs exert their toxic and carcinogenic effects. In the following paper the reactivity of PCB-quinoid metabolites toward nitrogen nucleophiles is extended to include their reactivity toward DNA bases and DNA itself.

4. Acknowledgements

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5. References

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